

# The effect of antioxidants on male factor infertility: the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial

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**Objective:** To determine whether antioxidants improve male fertility, as measured by semen parameters and DNA fragmentation at 3 months and pregnancy resulting in live birth after up to 6 months of treatment, among couples with male factor infertility.

**Design:** Multicenter, double-blind, randomized, placebo-controlled trial with an internal pilot study.

**Setting:** Nine fertility centers in the United States from December 2015 to December 2018.

**Patient(s):** Men (N = 174) with sperm concentration  $\leq 15$  million/mL, motility  $\leq 40\%$ , normal morphology  $\leq 4\%$ , or DNA fragmentation  $>25\%$ , and female partners who were ovulatory,  $\leq 40$  years old, and had documented tubal patency.

**Intervention(s):** Males randomly assigned to receive an antioxidant formulation (n = 85) containing 500 mg of vitamin C, 400 mg of vitamin E, 0.20 mg of selenium, 1,000 mg of L-carnitine, 20 mg of zinc, 1,000  $\mu$ g of folic acid, 10 mg of lycopene daily, or placebo (n = 86). Treatment lasted for a minimum of 3 months and maximum of 6 months, and couples attempted to conceive naturally during the first 3 months and with clomiphene citrate with intrauterine insemination of the female partner in months 4 through 6.

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**Main Outcome Measure(s):** Primary outcome was live birth; secondary outcomes included pregnancy within 6 months of treatment. For the internal pilot, the primary outcomes were semen parameters and sperm DNA fragmentation index after 3 months of treatment.

**Result(s):** In the Males, Antioxidants, and Infertility (MOXI) study, after 3 months of treatment, the change in sperm concentration differed between the antioxidant group (median  $-4.0$  [interquartile range  $-12.0, 5.7$ ] million/mL) and placebo group ( $+2.4$  [ $-9.0, 15.5$ ] million/mL). However, there were no statistically significant differences between the two groups for changes in sperm morphology, motility, or DNA fragmentation. Among the 66 oligospermic men at randomization, sperm concentration did not differ at 3 months between the antioxidant and control groups:  $8.5$  ( $4.8, 15.0$ ) million/mL versus  $15.0$  ( $6.0, 24.0$ ) million/mL. Of the 75 asthenospermic men, motility did not differ at 3 months:  $34\% \pm 16.3\%$  versus  $36.4\% \pm 15.8\%$ . Among the 44 men with high DNA fragmentation, DNA fragmentation did not differ at 3 months:  $29.5\%$  ( $21.6\%, 36.5\%$ ) versus  $28.0\%$  ( $20.6\%, 36.4\%$ ). In the entire cohort, cumulative live birth did not differ at 6 months between the antioxidant and placebo groups:  $15\%$  versus  $24\%$ .

**Conclusion(s):** Antioxidants do not improve semen parameters or DNA integrity among men with male factor infertility. Although limited by sample size, this study suggests that antioxidant treatment of the male partner does not improve in vivo pregnancy or live-birth rates.

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El resumen está disponible en Español al final del artículo.

**Key Words:** Antioxidants, male factor infertility, randomized controlled trial

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Antioxidants are currently being marketed to treat male factor infertility. Indeed, biologic evidence supports the hypothesis that antioxidants would improve male fertility. A variety of pathologic conditions may increase oxidative stress in semen (1–3). Oxidative stress can cause lipid peroxidation, thereby producing structural modifications to the sperm plasma membrane, which have been shown to interfere with sperm motility, acrosome reactions, and sperm-oocyte fusion (4). Oxidative stress may also damage the nuclear and mitochondrial genome by causing single and double DNA breaks, chemical modifications of bases, DNA crosslinks, and DNA protein crosslinks (5). In semen, antioxidants decrease oxidative stress (6), potentially improving sperm motility and reducing DNA fragmentation (7).

Studies of supplements have tended to show an improvement in semen parameters with the use of antioxidants. Benefits of vitamin E (8), selenium (9), N-acetylcysteine (10), or carnitine (7) on sperm motility have been seen after 3 months of treatment. Unfortunately, most of these studies have been small and heterogeneous. Although most of the studies included only infertile men, some included those with normal baseline semen parameters and some with abnormal baseline semen parameters. Treatment with vitamin C and vitamin E has been shown to reduce DNA fragmentation compared with placebo (11).

A recent meta-analysis concluded that antioxidant supplementation taken by subfertile males may increase the chance of live birth; however, large randomized, well-designed, placebo-controlled trials have been needed (7). A number of the included trials used antioxidants in combination with in vitro fertilization (IVF); it is certainly possible that the response to antioxidants would differ with IVF. In addition, the meta-analysis included trials of “substances with antioxidant properties” (myo-inositol, polyunsaturated acids, resveratrol, vitamin B, and vitamin D). A variety of antioxidant formulations are commercially available, but trials using antioxidant formulations have been limited by sample size and by

use of secondary end points. The Males, Antioxidants, and Infertility (MOXI) trial was designed to test the hypothesis that antioxidants would improve male fertility without the use of assisted reproductive technology (ART).

## MATERIALS AND METHODS

### Study Design

The MOXI clinical trial was conducted by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Cooperative Reproductive Medicine Network. The Collaborative Center for Statistics in Science at Yale University served as the data coordinating center. The trial was conducted at nine clinical sites throughout the United States.

A full description of the trial with inclusion and exclusion criteria is listed on [Clinicaltrials.gov](https://clinicaltrials.gov) (NCT02421887). This was a multicenter, randomized clinical trial involving couples with male factor infertility. Heterosexual couples with at least 12 months of infertility were eligible. Male partners were 18 years of age or older with at least one abnormal semen parameter on a semen analysis in the preceding 6 months: sperm concentration  $\leq 15$  million/mL (oligospermia), total motility  $\leq 40\%$  (asthenospermia), normal morphology  $\leq 4\%$  (teratospermia), or DNA fragmentation  $\geq 25\%$ . Female partners were between 18 and 40 years of age with regular menstrual cycles (defined as 25 to 35 days in duration), evidence of ovulation (by biphasic basal body temperature, ovulation predictor kits, or luteal serum progesterone level  $\geq 3$  ng/mL), and a normal uterine cavity with at least one patent fallopian tube. Women over the age of 35 had a normal ovarian reserve, defined as an early follicular phase follicle-stimulating hormone (FSH) level of  $\leq 10$  IU/L, an antimüllerian hormone (AMH) level of  $\geq 1.0$  ng/mL, or antral follicle count  $> 10$ . Male partners were excluded if they had a sperm concentration  $< 5$  million/mL on the screening semen analysis or if they were taking fertility medication or testosterone. Men were required to refrain from taking any vitamins for 4 weeks before randomization.

Approval for the study was obtained from the University of Pennsylvania, which served as the single institutional review board for each site, with additional local site review (12). Written informed consent was obtained from all male and female participants.

## Study Treatment

Men received a placebo or an antioxidant formulation containing 500 mg of vitamin C (ascorbic acid), 400 mg of vitamin E (d- $\alpha$  tocopheryl), 0.20 mg of selenium (L-selenomethionine), 1,000 mg of L-carnitine, 20 mg of zinc, 1,000  $\mu$ g of folic acid, 10 mg of lycopene, and 2,000 IU of vitamin D daily (IND #125753) for at least 3 months and up to 6 months. The antioxidant and placebo were purchased from and packaged by a commercial manufacturer for the study. This formulation was selected because it was commercially available and each component at comparable doses had been previously studied in a randomized, controlled trial and found to positively impact sperm structure or function and/or pregnancy rates after ART (13). The study medications were assigned in a double-blind fashion. The randomization scheme was generated using a computer-generated random number sequence in randomly varying blocks of four and six stratified by site and female age (<35 years and  $\geq$  35 years of age) with allocation 1:1 by the data-coordinating center through a Web-based, secured randomization service. Pill counts were conducted at each study visit to monitor compliance.

Male participants provided a semen sample on the day of randomization and after 90 days of treatment. The semen analysis included standard measurements such as volume, pH, count, and motility. Semen smears were prepared from each sample and shipped to the University of Utah School of Medicine Andrology and IVF Laboratory for centralized assessment of sperm morphology using World Health Organization 5.0 criteria. In addition, 1 mL of semen was stored at  $-80^{\circ}\text{C}$  and subsequently shipped frozen to the Utah Andrology Laboratory for DNA fragmentation assessment using the sperm chromatin structure analysis (SCSA) test (14), when 10 million sperm were present. A blood sample was obtained at randomization and after 3 months of treatment. The samples were shipped to ARUP Laboratories in Salt Lake City, UT, where they were analyzed for selenium, vitamin E- $\alpha$  tocopherol, vitamin E- $\gamma$  tocopherol, and zinc.

The couples were provided with free ovulation predictor tests and were instructed on timing their intercourse during the first 3 months of treatment (phase 1). Couples who had not conceived after 3 months of timed intercourse received up to three cycles of ovarian stimulation with clomiphene citrate with intrauterine insemination (phase 2). Women who conceived were observed through pregnancy and delivery.

## Outcomes

The primary outcome for the trial was live birth, defined as a delivery of a live infant after 20 weeks' gestation. The secondary outcomes included pregnancy, defined as a positive home pregnancy test, within 6 months of treatment. A prespecified, internal pilot was created to examine the effect of the antiox-

idant formulation on male semen parameters and DNA fragmentation at 3 months of treatment compared with controls. The protocol was designed such that if the pilot failed to reject the null hypothesis that motility and DNA fragmentation did not differ between the two treatment groups (antioxidant and placebo) after 3 months of treatment, the MOXI trial would stop enrollment.

## Statistical Analysis

The primary outcome was a live birth resulting from a pregnancy occurring within the 6 months of treatment. For the power analysis, a live-birth rate of 35% in the antioxidant group and 25% in the placebo group with a 17% dropout was assumed. A sample size of 395 in each group would yield 80% power using a two-sided chi-square test with  $\alpha=0.05$ . For the internal pilot, we assumed 50% of the males would have low motility (<40%) at baseline. For sample size calculations for the pilot study, we assumed that after 3 months of treatment sperm motility would differ by 13% (95% confidence interval [CI], 3.45%–23.49%) (13) between the antioxidant and placebo groups, and DNA fragmentation would be  $9.1\% \pm 7.2\%$  in the antioxidant group and  $22.1\% \pm 7.7\%$  placebo group (11). Assuming a 20% dropout rate, a sample size of 60 in each group would yield  $\geq 80\%$  power at  $\alpha=0.05$  for both outcomes.

Intention-to-treat analyses were performed to compare the two groups. Categorical data are reported as frequencies and percentages, and analysis conducted using chi-square analysis and Fisher's exact test where appropriate. Nonparametric data are expressed as median with interquartile range and bivariate analyses completed using the Wilcoxon rank sum test. Parametric data are expressed as mean  $\pm$  standard deviation (SD); Student's *t*-tests were used for analyses. Analyses were performed with SAS, version 9.4 (SAS Institute). A two-sided  $P < .05$  was considered statistically significant.

## RESULTS

We prescreened 822 couples. Of the 264 couples who provided written informed consent and completed the screening, 171 were eligible and were randomly assigned to a treatment group (Supplemental Fig. 1, available online), and 144 of these couples completed the study. The frequency of dropouts was not statistically significantly different between the study groups (21% in the antioxidant group, and 11% in the placebo group;  $P = .055$ ). Adherence, defined as intake of 80% or more of study drug during phase 1, was 88% among the antioxidant users and 82% among the placebo users ( $P = .26$ ).

Baseline characteristics are presented in Table 1 for the male participants and in Supplemental Table 1 (available online) for the female participants. The mean ( $\pm$  SD) selenium levels at randomization were  $160.3 \pm 19.8$   $\mu\text{g/L}$ , mean vitamin E- $\alpha$  tocopherol levels were  $9.6 \pm 2.7$   $\text{mg/L}$ , and mean zinc levels were  $89.3 \pm 12.1$   $\mu\text{g/dL}$ . Baseline characteristics were no different between the two groups, except men in the placebo group were more likely to have fathered a pregnancy in the past. The baseline semen characteristics (Table 2) were similar in the two groups, except men in the

TABLE 1

Characteristics at screening for all enrolled men in the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial.

Characteristics	Antioxidants (n = 85)	Placebo (n = 86)
Age (y)	34.0 (30.0, 38.0)	34.0 (30.0, 37.0)
Body mass index (kg/m <sup>2</sup> )	27.8 (24.2, 31.7), n = 82	27.6 (24.4, 31.0)
Ethnicity		
Hispanic or Latino	7 (8.2)	5 (5.8)
Non-Hispanic	72 (84.7)	78 (90.7)
Unknown	6 (7.1)	3 (3.5)
Race		
White	63 (74.1)	69 (80.2)
Black	6 (7.1)	7 (8.1)
Asian	7 (8.2)	2 (2.3)
American Indian or Alaska Native	1 (1.2)	1 (1.2)
Unknown	8 (9.4)	5 (5.8)
Mixed race	0 (0)	2 (2.3)
Abnormal semen parameters		
Single abnormal parameter		
Sperm concentration $\leq$ 15 million/mL	4 (4.7)	5 (5.8)
Total motility $\leq$ 40%	9 (10.6)	10 (11.6)
Normal morphology $\leq$ 4% <sup>a</sup>	33 (38.8)	29 (33.7)
>1 Abnormal parameters	39 (45.9)	42 (48.8)
Fathered a prior pregnancy <sup>b</sup>		
Yes	25 (29.4)	38 (44.2)
No	60 (70.6)	48 (55.8)
Prior infertility treatment and/or surgery		
Yes	25 (29.4)	24 (27.9)
No	60 (70.6)	62 (72.1)
Duration of infertility (mo)	24.0 (18.0, 48.0), n = 81	24.0 (15.0, 36.0), n = 83
History of smoking		
Never	54 (63.5)	47 (54.7)
Current	8 (9.4)	11 (12.8)
Former	23 (27.1)	28 (32.6)
History of alcohol use		
Never	6 (7.1)	4 (4.7)
Current (in past year)	72 (84.7)	81 (94.2)
Former (not in past year)	7 (8.2)	1 (1.2)

Note: Data are presented as the number (%) or median (interquartile range).

<sup>a</sup> Normal based on WHO 5th criteria.

<sup>b</sup>  $P < .05$ , Wilcoxon rank sum test was used for the continuous variables, and chi-square or Fisher's exact test was used for categorical variables. Wilcoxon rank sum test was used to test the distributional difference, instead of mean or median of the two groups.

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antioxidant group had a lower percentage of morphologically normal sperm.

Changes in semen parameters between baseline and month 3 of treatment are presented in Table 3. The changes in sperm concentration, total sperm count, and total motile sperm count were statistically significantly different between the two groups, with an increase in the placebo group and a decline in the antioxidant group. Morphology, motility, and DNA fragmentation did not change between the two groups. Selenium, vitamin E- $\alpha$  tocopherol, and zinc levels increased after 3 months of treatment in the antioxidant group but did not change in the placebo group. Vitamin E- $\gamma$  tocopherol levels did not change in either group (Supplemental Table 2,

available online). (Note that the antioxidant formulation contained vitamin E- $\alpha$  tocopherol and did not contain vitamin E- $\gamma$  tocopherol.)

The changes in semen parameters between baseline and month 3 of treatment between the two treatment groups (antioxidant and placebo) for the subgroups of men with oligospermia, asthenospermia, teratospermia, and high DNA fragmentation are presented in Table 4. Among the 66 men with oligospermia, there were no statistically significant changes in sperm concentration between the two treatment groups. Of the 48 men with teratospermia, there were no statistically significant changes in normal sperm morphology between the two treatment groups. Among the 75 men with asthenospermia, there were no statistically significant changes in sperm motility between the two treatment groups. There also were no significant changes in DNA fragmentation between the two treatment groups among the 44 men with high DNA fragmentation at baseline.

Because we failed to reject the null hypothesis for the internal pilot, further enrollment in the trial was stopped based on the recommendation of the data and safety monitoring board; all enrolled couples completed the study protocol. Fifteen percent (13 of 85) of the couples whose male partner had received antioxidants had a live birth, compared with 24% (21 of 86) of those randomized to the placebo ( $P = .14$ ). Pregnancy rates in the antioxidant group and in the placebo group did not differ in phase 1 (9% vs. 9%,  $P = .98$ ) when couples received no additional treatment, or in phase 2 (12% vs. 21%,  $P = .11$ ) when women received ovarian stimulation with clomiphene citrate and timed intrauterine insemination. First-trimester pregnancy loss did not differ between groups (22% vs. 19%,  $P = 1.0$ ). Similar results were observed when the male participants were stratified based on baseline sperm morphology or prior pregnancy history (Supplemental Table 3, available online). Serious adverse events were not observed among any of the male participants. The percentage of men who had at least one adverse event did not differ between the groups (41% in antioxidant group and 40% in placebo group,  $P = .83$ ) (Supplemental Table 4, available online). The pregnancy and live-birth rates also did not differ between the groups in a per protocol analysis (Supplemental Table 5, available online).

## DISCUSSION

In this randomized controlled trial of couples with male factor infertility, the use of an antioxidant combination in the male partner did not result in a statistically significant improvement in semen parameters after 3 months of therapy compared with placebo. Furthermore, men with asthenospermia or high DNA fragmentation did not exhibit an improvement in motility or a decrease in DNA fragmentation, as had been hypothesized. Although the internal pilot was not powered to examine differences in pregnancy rates, couples whose male partner received an antioxidant were not more likely to conceive during natural cycles or with intrauterine insemination.

Treatment with an antioxidant formulation did not increase motility among the entire cohort nor in the subgroup



TABLE 2

Semen parameters at randomization in the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial.

Parameters	Antioxidants (n = 85)	Placebo (n = 86)
Sperm concentration (million/mL)	21.0 (11.0, 41.2)	16.7 (10.4, 42.0)
Sperm concentration $\leq 15$ million/mL	31 (36.5)	39 (45.4)
Normal morphology (%) <sup>a</sup>	4.0 (2.0, 8.0), n = 63	6.0 (3.0, 10.0), n = 63
Normal morphology $\leq 4\%$ <sup>a</sup>	33 (52.4)	19 (30.2)
Total motility (%)	44.9 $\pm$ 17.3	43.0 $\pm$ 15.7
Total motility $\leq 40\%$	36 (42.4)	43 (50.0)
DNA fragmentation (SCSA, DFI) (%)	18.7 (14.3, 28.3), n = 73	21.1 (14.1, 28.6), n = 74
DNA fragmentation $>25\%$	23 (31.5)	26 (35.1)
Total sperm count (million)	47.6 (24.7, 84.0)	53.4 (26.4, 90.0)
Total motile sperm count (million)	20.7 (7.4, 44.5)	23.4 (8.6, 46.7)

Note: Data are presented as median (interquartile range), mean ( $\pm$  standard deviation) or number (%). DFI = DNA fragmentation index; SCSA = sperm chromatin structure analysis.

<sup>a</sup>  $P < .05$ . Student's *t*-test or Wilcoxon rank sum test was used for continuous variables; chi-square test was used for categorical variables.

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with asthenospermia at baseline. Although benefits of vitamin C (15), selenium (9), N-acetylcysteine (10), L-carnitine (16), and zinc (17) on sperm motility have been found after 3 months of treatment, most of those studies were small and heterogeneous. Most studies have included only infertile men, although some included men with normal baseline semen parameters and some with abnormal baseline semen parameters. A recent Cochrane meta-analysis by Smits et al. (7), which included only with men with abnormal sperm, found that only N-acetylcysteine, selenium, and vitamin E alone improved sperm motility. Given the degree of heterogeneity, pooling of all antioxidant results was not possible. However, the Cochrane meta-analysis did find a 12% absolute increase in motility in men treated with combination antioxidants for 3 months compared with controls (7). In the trial by Raigani et al. (18), 84 men with oligoasthenoteratospermia using a combination of folic acid and zinc for 14 weeks showed no improvement in sperm motility after 14 weeks of therapy, similar to our findings.

Treatment with an antioxidant formulation did not decrease DNA fragmentation as measured by the SCSA among the entire cohort or among men with high DNA fragmentation at baseline. Only a few clinical trials have compared sperm DNA fragmentation between those treated with and without antioxidants. Greco et al. (11) enrolled 64 men with DNA fragmentation levels  $\geq 15\%$ , as measured using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. After 2 months of treatment with vitamin E and vitamin C, the DNA fragmentation index decreased from 22% to 9%, with no change noted in the placebo group. Although the DNA fragmentation levels were no different in our cohort at baseline, we did not observe a similar reduction over 3 months, despite using a combination that included both vitamin E and C. Our cohort was over twice the size, and we used SCSA, not TUNEL, to quantify DNA fragmentation. Just as we noted in men with high DNA fragmentation ( $>25\%$ ), Stenqvist et al. (19) found no improvement in DNA fragmentation as measured by SCSA after 6

TABLE 3

Change in semen parameters from baseline to month 3 in the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial.

Parameters	Antioxidants		Placebo		P value <sup>a</sup>
	n	Value	n	Value	
Sperm concentration (million/mL)	82	-4.0 (-12.0, 5.7) <sup>b</sup>	82	2.4 (-9.0, 15.5)	.029
Normal morphology (%)	55	0 (-2.0, 1.0)	55	0 (-2.0, 1.0)	.470
Total motility (%)	82	-1.6 $\pm$ 16.0	82	-1.1 $\pm$ 13.7	.822
DNA fragmentation (SCSA, DFI) (%)	65	0.8 (-3.4, 3.8)	70	0.2 (-5.7, 6.4)	.548
Total sperm count (million)	82	-10.6 (-32.5, 12.6) <sup>b</sup>	82	1.6 (-21.8, 42.9)	.021
Total motile sperm count (million)	82	-4.0 (-13.2, 9.9)	82	1.5 (-11.8, 15.4)	.043

Note: Data are presented as median (interquartile range) or mean ( $\pm$  standard deviation). DFI = DNA fragmentation index; SCSA = sperm chromatin structure analysis.

<sup>a</sup> Comparison of change between antioxidants and placebo groups, Student's *t*-test or Wilcoxon rank sum test.

<sup>b</sup> Statistically significant change ( $P < .05$ ) from baseline to month 3.

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TABLE 4

Semen parameters from baseline to month 3 by sperm abnormality subgroups at baseline in the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial.

Baseline semen parameters in groups (nonexclusive)	Visit 3 values					Change of parameter					P value <sup>a</sup>
	Antioxidant group			Placebo group		Antioxidants group		Placebo group			
	N	n	Value	n	Value	n	Value	n	Value		
Sperm concentration ≤15 million/mL	66	31	8.5 (4.8, 15.0)	35	15.0 (6.0, 24.0)	31	3.0 (−3.0, 9.0) <sup>b</sup>	35	7.0 (−2.0, 14.9) <sup>c</sup>	.298	
Normal morphology ≤4%	48	30	2.0 (1.0, 5.0)	18	2.5 (2.0, 4.0)	30	0 (−1.0, 2.0)	18	0.3 (−1.0, 2.0) <sup>b</sup>	.863	
Total motility ≤40%	75	35	34.0 ± 16.3	40	36.4 ± 15.8	35	5.1 ± 16.1	40	5.1 ± 13.9 <sup>b</sup>	.929	
DNA fragmentation (SCSA, DFI) >25%	44	19	29.5 (21.6, 36.5)	25	28.0 (20.6, 36.4)	19	−2.0 (−6.6, 3.7)	25	−6.5 (−12.5, 0.7) <sup>b</sup>	.197	

Note: Data are presented as median (interquartile range) or mean ( $\pm$  standard deviation). DFI = DNA fragmentation index; SCSA = sperm chromatin structure analysis.

<sup>a</sup> Comparison of change between antioxidants and placebo groups, Student's *t*-test or Wilcoxon rank sum test.

<sup>b</sup> Statistically significant change ( $P < .05$ ) from baseline to month 3.

<sup>c</sup> Statistically significant change ( $P < .01$ ) from baseline to month 3.

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months of therapy with an antioxidant combination. The recent Cochrane meta-analysis, which included five trials with a variety of antioxidants with a total of 254 participants, found that men treated with antioxidants had on average 5% lower DNA fragmentation, but the confidence interval was broad and crossed 0 (7). Taken together with previous findings, our results indicate that while antioxidants may reduce reactive oxygen species (ROS), this does not appear to translate into reduced sperm DNA fragmentation.

Couples in which the male partner was treated with antioxidants were not more likely to have a pregnancy resulting in a live birth in the first 3 months of treatment with timed intercourse, nor in the second 3 months of treatment with ovarian stimulation with intrauterine insemination. Antioxidants did not improve pregnancy or live-birth rates.

Given that our trial also found no improvement in semen parameters, the data and safety monitoring board concluded that continuing the trial in light of the lack of response was not justifiable. The trial-stopping rule had the strong underlying hypothesis that the effect of the intervention on live births is (at least partially) mediated through improvements in sperm motility and a reduction in sperm DNA fragmentation. The internal pilot study was designed to provide further evidence that antioxidants could improve semen quality to justify an investment in a trial of sufficient magnitude to study the outcome of live birth. However, conventional semen quality parameters and even sperm DNA fragmentation are, at best, modest predictors of a couple's fertility when trying with or without medical assistance.

The recent Cochrane Review found that antioxidant use increased the odds of pregnancy by 2.97-fold and the odds of live birth by 1.8-fold (7). The meta-analysis included nine studies of six antioxidant or antioxidant combinations for a total of 750 participants in the live-birth analysis. Two of the trials, which strongly favored antioxidants, were in couples undergoing IVF (20, 21). Follow-up evaluation in the natural conception trials was not systematic (22, 23). In the Omu trial (23), the couples were evaluated for 6 months after cessation of antioxidant therapy. Similar to our MOXI

trial, the high-quality trials included in the Cochrane review did not find a benefit to antioxidants on live birth (24, 25).

Our negative findings contradict the overall conclusions from the Cochrane Review and meta-analysis. This could be due to many factors. Henkel et al. (26) suggest that excessive use of antioxidants may upset the balance between oxidation and reduction, leading to reductive stress. Although this is a theoretical concern, the antioxidant formulation used in our study did not include excessive amounts of any given antioxidant; the doses aligned with those used in prior trials.

The antioxidant formulation was selected based on input from the steering committee, advisory board, and data and safety monitoring board. Although one or two individual antioxidants could have been selected for the study, a combination formulation was selected because [1] there are multiple antioxidants, [2] antioxidant formulations are being marketed and prescribed, and [3] there was no single "superior" antioxidant. A commercially available antioxidant formulation was selected to reduce the potential for opposing effects of antioxidants, reductive stress due to excessive antioxidants, or poor or impure product selection. Unfortunately, the design of the MOXI trial does not allow the differentiation of effects of individual nutrients and inherently assumes there are no interacting effects between the different antioxidants in the formulation. Because this assumption may not be true, future randomized controlled trials could study individual components through a factorial design.

Another theoretical concern is that we selected patients who would be unlikely to benefit from antioxidants. For example, only men with elevated levels of ROS should have been included. However, this is not how antioxidants are currently marketed or prescribed. Our inclusion criteria were similar if not more selective compared with prior trials. We also evaluated subgroups who were more likely to have ROS damage—those with asthenospermia and high DNA fragmentation—and did not see any evidence of benefit.

This multisite, randomized, double-blind, placebo-controlled trial was designed with adequate power to

determine the extent to which antioxidants improve semen parameters and DNA fragmentation. Prior trials have been small and of low or very low quality (13). All men enrolled in the MOXI trial had male factor infertility, with at least one abnormal semen parameter and a partner with normal fertility testing. Plasma vitamin levels confirm that men in the antioxidant group complied with the regimen, and the men randomized to placebo did not cross over. The trial was powered to examine changes in semen parameters in the entire cohort and in subgroups with specific sperm abnormalities.

Although MOXI was not powered to determine group differences in live birth, it is the largest, appropriately designed trial to date to examine the impact of antioxidant treatment in the male partner on subsequent non-ART outcomes; we found no increase in live birth either with timed intercourse or with intrauterine insemination. Future studies may seek to determine whether there are subpopulations (e.g., men with low vitamin levels, men with high levels of ROS in their semen) for which antioxidants may improve semen parameters. Larger trials are needed to examine live birth as an outcome.

## CONCLUSION

Antioxidant treatment does not improve semen parameters or DNA integrity in infertile males. Although limited by sample size, this study suggests that combination antioxidant treatment of the male partner does not improve in vivo pregnancy or live-birth rates in couples with male factor infertility, but larger trials are needed to confirm this finding.

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**El efecto de los antioxidantes en el factor masculino de infertilidad: ensayo clínico aleatorizado de hombres, antioxidantes e Infertilidad**

**Objetivo:** Determinar si los antioxidantes mejoran la fertilidad masculina, según lo medido por los parámetros de semen y fragmentación del ADN a los 3 meses, y embarazo resultante en un nacido vivo después de 6 meses de tratamiento en parejas con infertilidad por factor masculino.

**Diseño:** Ensayo multicéntrico, doble ciego, aleatorizado, controlado con placebo con un estudio piloto interno.

**Lugar:** Nueve centros de fertilidad en los Estados Unidos desde diciembre del 2005 hasta diciembre del 2018.

**Pacientes:** Hombres (n= 174) con concentración de esperma  $\leq 15$  millones/ml, movilidad  $\leq 4\%$ , morfología normal  $\leq 4\%$ , o fragmentación del ADN  $> 25\%$  y parejas femeninas las cuales eran ovulatorias,  $\leq 40$  años y tenían permeabilidad tubárica documentada.

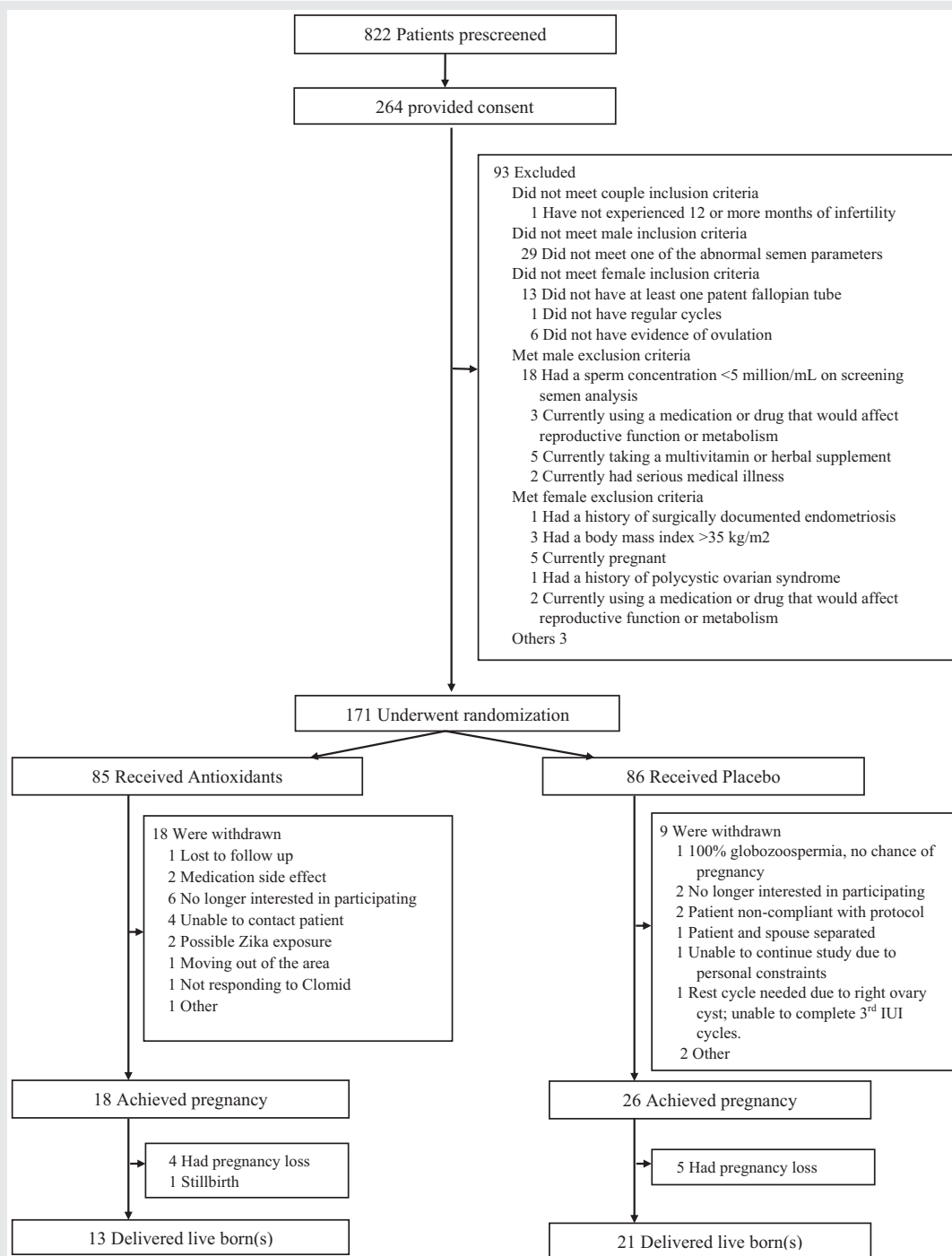
**Intervenciones:** Hombres asignados al azar para recibir una formulación antioxidante (n = 85) que contiene 500 mg de vitamina C, 400 mg de vitamina E, 0.20 mg de selenio, 1.000 mg de L-carnitina, 20mg de zinc, 1.000 ug de ácido fólico, 10 mg de licopeno diario o placebo (n= 86). El tratamiento duró un mínimo de 3 meses y un máximo de 6 meses y las parejas intentaron concebir naturalmente durante los primeros 3 meses y con citrato de clomifeno con inseminación intrauterina de la pareja femenina en los meses 4 a 6.

**Principales medidas de resultado:** El primer resultado fue el nacimiento vivo, los segundos resultados incluyeron embarazo dentro de los 6 meses de tratamiento. Para el piloto interno, el primer resultado fueron los parámetros de semen e índice de fragmentación del ADN espermático después de 3 meses de tratamiento.

**Resultados:** En el estudio Masculinos, Antioxidantes e Infertilidad (MOXI), después de 3 meses de tratamiento, el cambio en la concentración de esperma difirió entre el grupo antioxidante (mediana -4,0 [rango intercuartil -12,0- 5,7] millones / mL) y el grupo placebo (+2,4 [9,0- 15,5] millones / mL). Sin embargo, no hubo diferencias estadísticamente significativas entre los dos grupos para los cambios de morfología, motilidad o fragmentación del ADN espermático. Entre los 66 hombres oligospermicos aleatorizados, la concentración de esperma no difirió a los 3 meses entre los grupos antioxidante y control: 8,5 (4,8- 15,0) millones / mL versus 15,0 (6,0- 24,0) millones / mL. De los 75 hombres astenospermicos, la motilidad no difirió a los 3 meses:  $34\% \pm 16,3\%$  versus  $36,4\% \pm 15,8\%$ . Entre los 44 hombres con alta fragmentación del ADN, la fragmentación del ADN no difirió a los 3 meses:  $29,5\%$  (21,6% - 36,5%) versus  $28,0\%$  (20,6% - 36,4%). En toda la cohorte, la tasa acumulada de nacido vivo no difirió a los 6 meses entre los grupos de antioxidantes y placebo: 15% versus 24%.

**Conclusiones:** Los antioxidantes no mejoran los parámetros de semen o integridad del ADN entre los hombres con infertilidad por factor masculino. Aunque limitado por el tamaño muestral, este estudio sugiere que el tratamiento de la pareja masculina con antioxidantes no mejora las tasas de embarazo o nacido vivo.

## SUPPLEMENTAL FIGURE 1



Study flow chart of enrollment and outcomes of the trial.

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## SUPPLEMENTAL TABLE 1

## Characteristics of women at screening in the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial.

Characteristics	Antioxidants (n = 85)	Placebo (n = 86)
Age (y)	31.0 (29.0, 35.0)	32.0 (29.0, 35.0)
Body mass index (kg/m <sup>2</sup> )	23.8 (21.6, 27.7)	24.2 (22.2, 27.7) <sup>a</sup>
Ethnicity		
Hispanic or Latino	5 (5.9)	7 (8.1)
Non-Hispanic	77 (90.6)	76 (88.4)
Unknown	3 (3.5)	3 (3.5)
Race		
White	69 (81.2)	67 (77.9)
Black	6 (7.1)	7 (8.1)
Asian	8 (9.4)	5 (5.8)
American Indian or Alaska Native	1 (1.2)	0 (0.0)
Native Hawaiian or Other Pacific Islander	0 (0)	1 (1.2)
Unknown	0 (0)	2 (2.3)
Mixed race	1 (1.2)	4 (4.7)

Note: Data are presented as the number (%) or median (interquartile range). There were no statistically significant differences ( $P < .05$ ) between the two groups.

<sup>a</sup> n = 83.

## SUPPLEMENTAL TABLE 2

## Plasma vitamin levels by treatment group in the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial.

Visit	Items	Group	N	Mean	Standard deviation	Median	25th Percentile	75th Percentile
Randomization (visit 1)	Selenium ( $\mu\text{g/L}$ )	Total	124	160.3	19.8	156.5	146.0	174.0
		Antioxidant	62	158.2	19.1	154.5	143.0	170.0
		Placebo	62	162.4	20.4	160.0	148.0	176.0
	Vitamin E- $\alpha$ tocopherol (mg/L)	Total	131	9.6	2.7	9.5	8.0	10.7
		Antioxidant	64	9.7	2.8	9.6	8.1	11.1
		Placebo	67	9.6	2.7	9.5	7.8	10.5
	Vitamin E- $\gamma$ tocopherol (mg/L)	Total	131	1.3	0.7	1.2	0.8	1.7
		Antioxidant	64	1.4	0.7	1.2	1.0	1.7
		Placebo	67	1.2	0.6	1.1	0.8	1.7
	Zinc ( $\mu\text{g/dL}$ )	Total	128	89.3	12.1	88.0	81.5	96.0
		Antioxidant	65	89.5	11.7	88.0	82.0	96.0
		Placebo	63	89.1	12.6	88.0	80.0	96.0
After 3 months of treatment (visit 3)	Selenium ( $\mu\text{g/L}$ )	Total	124	189.6	37.1	186.0	156.5	219.5
		Antioxidant	62	217.5	27.5	218.0	194.0	235.0
		Placebo	62	161.6	20.6	156.5	147.0	172.0
	Vitamin E- $\alpha$ tocopherol (mg/L)	Total	131	12.1	4.4	11.4	8.9	14.2
		Antioxidant	64	14.8	4.3	14.0	12.0	16.7
		Placebo	67	9.4	2.5	9.1	7.6	10.6
	Vitamin E- $\gamma$ tocopherol (mg/L)	Total	131	0.9	0.6	0.8	0.5	1.2
		Antioxidant	64	0.6	0.3	0.6	0.3	0.8
		Placebo	67	1.2	0.6	1.2	0.8	1.4
	Zinc ( $\mu\text{g/dL}$ )	Total	128	90.6	14.2	89.0	82.5	98.0
		Antioxidant	65	93.4	13.6	92.0	86.0	102.0
		Placebo	63	87.7	14.3	85.0	80.0	94.0
Visit 3–visit 1	Selenium ( $\mu\text{g/L}$ )	Total	124	29.3	34.4	27.0	−0.5	60.0
		Antioxidant	62	59.3	20.1	60.0	44.0	72.0
		Placebo	62	−0.8	12.3	−0.5	−10.0	5.0
	Vitamin E- $\alpha$ tocopherol (mg/L)	Total	131	2.4	3.5	1.5	−0.4	4.6
		Antioxidant	64	5.1	2.9	4.7	3.4	6.8
		Placebo	67	−0.2	1.4	−0.2	−1.1	0.8
	Vitamin E- $\gamma$ tocopherol (mg/L)	Total	131	−0.4	0.7	−0.4	−0.8	0.1
		Antioxidant	64	−0.8	0.6	−0.7	−1.1	−0.4
		Placebo	67	0.0	0.5	0.0	−0.3	0.2
	Zinc ( $\mu\text{g/dL}$ )	Total	128	1.3	12.6	1.5	−5.5	8.0
		Antioxidant	65	3.9	12.1	4.0	−4.0	10.0
		Placebo	63	−1.4	12.7	−1.0	−9.0	5.0

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## SUPPLEMENTAL TABLE 3

Live-birth rate by sperm morphology category or male prior pregnancy history status in the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial.

Category	Antioxidants	Placebo	P value
Normal morphology			
≤4%	3/33 (9.1)	5/19 (26.3)	.124
>4%	7/30 (23.3)	13/44 (29.6)	.555
Missing	3/22 (13.6)	3/23 (13.0)	1.000
Fathered a prior pregnancy			
Yes	2/25 (8.0)	9/38 (23.7)	.176
No	11/60 (18.3)	12/48 (25.0)	.400

Note: Data are presented as number/total number (%).

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## SUPPLEMENTAL TABLE 5

Pregnancy outcomes by treatment group (per protocol analysis) in the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial.

Outcomes	Antioxidants	Placebo	P value
Live birth	13/67 (19.4)	21/77 (27.3)	.267
Singleton live birth	11/67 (16.4)	16/77 (20.8)	.504
Twin or triple live birth	2/67 (3.0)	5/77 (6.5)	.450
Conception	18/67 (26.9)	26/77 (33.8)	.370
Phase 1	8/67 (11.9)	8/77 (10.4)	.768
Phase 2	10/67 (14.9)	18/77 (23.4)	.201
Clinical pregnancy	15/67 (22.4)	22/77 (28.6)	.397
Pregnancy loss	4/18 (22.2)	5/26 (19.2)	1.000

Note: Data presented as number/total number (%).

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## SUPPLEMENTAL TABLE 4

Adverse events and serious adverse events according to treatment groups in the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial.

Events	Antioxidants (n = 85)	Placebo (n = 86)	P value
Overall adverse events for couples <sup>a</sup>			
≥ 1 Serious adverse event	4 (4.7)	6 (7.0)	.746
≥ 1 Adverse event	51 (60.0)	59 (68.6)	.240
Adverse events for males <sup>b</sup>			
Abdominal pain	0 (0)	4 (4.7)	.121
Dyspepsia	4 (4.7)	2 (2.3)	.443
Headache	15 (17.6)	7 (8.1)	.063
Nasopharyngitis	4 (4.7)	7 (8.1)	.535
Nausea	1 (1.2)	4 (4.7)	.368
Upper respiratory infection	4 (4.7)	4 (4.7)	1.000

Note: Data are presented as the number (%).

<sup>a</sup> Including any events from men, women, or fetus/infant.

<sup>b</sup> Including adverse events that were observed in at least 4% of men in any of the treatment groups.

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